

Thermodynamic Stability and Stacking Interactions Are Reduced by replacement of Adjacent Purines by Pyrimidines at an Abasic Site in 15-mer DNA Duplexes.

János Sági, Anton B. Guliaev and B. Singer\*

Abasic site (AP)-containing duplexes, in which the AP site was flanked by adenine (A) or cytosine (C) bases had been shown to be more stable with flanking A than with C bases [J. Sági, B. Hang and B. Singer (1999) *Chem. Res. Toxicol.* 12, 917-923]. In this work we investigated whether the smaller destabilization by an AP site, when the neighbors are A, is a general effect of the purine neighbors *versus* the pyrimidines. An AP site (x), flanked by symmetric doublet bases, was first incorporated opposite T into 15-mer duplexes. Duplex stability was markedly decreased by the AP site in all cases, as compared to the corresponding control duplexes. The largest destabilization was observed with the duplex containing a central -TTxTT- sequence in which the  $\Delta T_m$  was -19.4°C and the  $\Delta\Delta G_{37}^\circ$  was -6.57 kcal/mol. Changing the central -TTxTT- to the -AAxAA- sequence resulted in a smaller destabilization (-17.3 °C and -5.65 kcal/mol), although the neighbors were A•T base pairs in both cases. Similarly, the AP site in -GGxGG- sequence proved to be less destabilizing (-13.9°C, -5.74 kcal/mol) than in the -CCxCC- sequence (-15.8°C, -7.39 kcal/mol) although G.C pairs flanked the AP site in both cases. The same differential destabilization was also observed with duplexes that contained an A or C opposite the AP site. The average stabilizing effect of the symmetric doublet purine neighbors of an AP site opposite a T, A or C was 3.2°C  $\Delta T_m$  and 1.3 kcal/mol  $\Delta\Delta G_{37}^\circ$ , as compared to the pyrimidine neighbors. The opposite G base reduced or eliminated the differential effect of the neighbors. Unrestrained molecular dynamics was used to evaluate the effect of the flanking sequences on structural features of the duplexes containing T opposite the AP site. Explicit solvent and the particle mesh Ewald method were applied for accurate representation of the electrostatic interactions. The duplexes with doublet pyrimidine neighbors showed a larger magnitude of curvature around the lesion site than did the duplexes with the purines flanking the AP site. Structural analysis showed that the purines flanking the AP site tend to shift toward each other, creating overlap, contrary to the flanking pyrimidines. This indicates the possibility of stacking between purine bases at the AP site and can be the reason for the observed smaller thermodynamic destabilization of the duplexes with the -AAxAA- and -GGxGG- central sequences, as compared to those with -TTxTT- and -CCxCC- sequences. The data also suggest that, similar to other base lesions, a high GC-content, compared to the high AT content, showed less destabilization of the duplex by the AP site. This work demonstrates that for an AP site the GC-content is not the only determinant of duplex stability, but rather is influenced more by whether purines or pyrimidines flanked the AP site.